Polyembryony in Parasitic Hymenoptera: A Review.

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With Plates 14 and 15.

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Introductory.

Among the specially remarkable facts in the study of embryology that of polyembryony or germinogony in Parasitic Hymenoptera is not the least noteworthy. Polyembryony consists in the production from one single egg, by a process of gemmation, of a large number of separate embryos. Polyembryonic species of Hymenoptera are known from the Chalcididæ and the Proctotrypidæ; many of these insects are jet

black or brownish creatures about 1 or 2 mm. in length, with gaudily sheeny wings and bodies; in many cases they are so small as to be almost invisible to the naked eye, in others these insects are much larger, but British species above 5 mm. in length are not common.

In connection with the word Hymenoptera one naturally associates thoughts of the wonderful habits and life-histories of Ants, Bees, and Wasps, but among such parasitic forms as the Chalcids one finds instincts and wonderful life-histories just as remarkable as in other forms. Within the last decade a great many new facts have been ascertained with regard to polyembryony, principally by Marchal (**5**), Silvestri (**9**), Martin (**14**), and Patterson (**13**).

Bionomical.

Holometabolous insecta from several orders are known to be the hosts of polyembryonic Hymenoptera, but the majority of the latter parasitise moth or butterfly larvæ. Pieris brassicæ is a martyr to all kinds of hymenopterous parasites.

Prof. Martelli (**17**) has given a list containing fifteen parasites and fourteen hyperparasites on this common Lepidopteron, and of course all such parasites have a value from an economic viewpoint. In the Oxford district Apantales glomeratus[1] is responsible for the slaughter of thousands of Pierid caperpillars, while a hyperparasite Mesochorus pallidus takes a large toll on the parasitic Apantales. In the small extent of a little cabbage-patch one then realises that wars and counter-wars are raging between the various parasitic fauna. The polyembryonic parasite seeks among the leaves of the food-plant of the host caterpillar for eggs of its host. The Hymenopteron alighting on these eggs pierces some of them with its ovipositor and lays in them one or more of its eggs. This is shown in Pl. 14, figs. 1 and 2, *B*. Each egg eventually gives rise to many larvæ.

The parasite's egg (or eggs) generally lies inside the sub-

[1] Monembryonic

stance of the host egg. The latter segments as usual and eventually the small moth larva emerges from the egg. Outwardly the host larva resembles others of its kind, but there is this difference—inside its body lies a small germinal mass derived from the parasite's egg, which later breaks up into a very large number of embryonic masses or gemmules, which give rise to larvæ, and which are destined later to destroy the unfortunate host larva.

If a parasitised larva nearly full-grown be opened up, it will be found to contain a large number of parasitic larvæ which have been nourished at the expense of the host tissue. These larvæ may be all derived from the one egg, laid by a parasite inside the egg laid by a moth or butterfly. The purpose of this short paper is to describe how this process is brought about.

After the parasitised larva has grown a good deal, in some cases when the observer is well acquainted with the behaviour of the healthy non-parasitised larvæ, certain differences in the reactions of the older parasitised larvæ can be detected. Such larvæ may often be found to be "sleepy," that is, they are not so active as their healthy fellows; moreover, in certain cases where the host larva is delicately tinted in transparent colours, parasitised specimens can easily be found, for the fat body which, in thin-skinned larvæ causes a definite appearance, is in these cases altered or absent.

The parasitised larva in later stages of its life is merely an empty skin full of parasites.

Generally everything except brain, etc., gut, and absolutely necessary organs are eaten up; a noteworthy fact is that the tracheal tubes are left quite intact. In fact, the parasites eat up everything but that absolutely necessary to keep their "vache à lait" living.

When the parasites have used their unfortunate host long enough to enable them to grow sufficiently they either kill their host by eating up its vitals or by boring holes in its sides in order to emerge from its body.

They now pupate near or inside the skin of their host.

Specimen Description of the Life-cycles of Host and Parasite (from Marchal).

Hyponomeutus mahalebellus is a moth whose caterpillar is the host of the parasite, Encyrtus fuscicollis, the polyembryonic Hymenopteron.

(1) The moths emerge from their cocoons in the last days of July.

(2) Encyrtus fuscicollis commence to emerge early in August.

(3) Hyponomeutus lays its eggs early in August, and the Encyrtids by August 15th are at their busiest parasitising these eggs. By August 22nd the parasites are rare.

(4) The parasitised eggs segment and develop, and by the end of September the contained moth embryos are ready to hatch.

The young larvæ do not emerge till next spring, but winter under cover of their egg cases.

(5) By spring the moth larvæ awaken, and the parasite's eggs contained in their bodies begin to develop. The trophoamnion is formed (see p. 180).

(6) By the end of spring the parasitic polygerminal mass is well formed (Text-figs. 11 and 12), and the host cyst (Kyste adventice of Marchal) or covering of host-cells around the parasitic mass is completely differentiated (see p. 183).

(7) By June or July the gemmules have metamorphosed into larvæ; the latter have broken out of their sheaths and the parasitised Lepidopterous larva is killed and its viscera eaten up (see p. 186).

(8) Encrytids pupate and finally emerge in August in time to parasitise other moth eggs.

With regard to the numbers of individuals produced from one host caterpillar, Howard ('Pr. U.S. Nat. Mus.,' xiv), affirms that in the case of Plusia brassicæ 2500 Litomastix truncatellus were hatched out of one caterpillar carcase. Giard ('Ann. Soc. Ent.,' Fr., 1898), writes that he

found nearly 3000 individuals from one larva of Plusia. Of course not all these arose from one Litomastix egg (**9**). Patterson (**13**) got on the average 175 individuals of Copidosoma from one Gnorimochema larva; the number from one caterpillar carcase varied from 25 to 395.

Early Development of the Egg of the Polyembryonic Species Ageniaspis (Encyrtus) fusicollis (Dalm.), parasite of Hyponomeutus malinellus or Hyponomeuta cognatella, etc. Marchal (**5**), Martin (**14**), Silvestri (**9**).

The newly-laid egg resembles that of the majority of parasitic Hymenoptera. The nucleus is of the condensed type. If the egg has been fertilised it contains a solid, bluntly comet-shaped spermatozoon. There is also the germ-cell determinant. The solid nucleus now breaks up into chromosomes and the first polar body forms. The spindle of this does not appear to have the usual centrosomes at its poles. The polar figure generally lies towards the upper central region of the egg. The oocyte nucleus again divides to form the second polar body, and at the same time the first polar body itself enters into division stages. Pl. 14, fig. 5: In this figure both spindles are in telophase, the sperm lies near the second polar body spindle, and the germ-cell determinant takes up its position in the interior of the lower part of the egg.

After the extrusion of the second polar body the matured egg nucleus assumes the resting reticulate appearance, while the three polar bodies (the first one divided) lie in the upper part of the egg in the form of three condensed solid plates, Pl. 14, fig. 6: If the egg was fertilised, the sperm, as is usually the case, breaks into a reticulum and takes its place next the egg nucleus. Pl. 14, fig. 6: The germ-cell determinant keeps closely in this region (*G.C.D.*). When the zygote nucleus is formed (fertilised egg), or when the matured egg nucleus resumes its reticulate shape (unfertilised egg), a most remark-

able process begins ; a definite region of the cytoplasm of the egg round the segmentation nucleus becomes separated off from the rest of the egg, Pl. 14, fig. 7. This region contains the segmentation nucleus and the germ-cell determinant (*G.C.D.*).

The egg now contains two distinct regions :

(1) The embryonic region with the nucleus and the germ-cell determinant (Embryonic ooplasm).

(2) The polar ooplasm so-called, with the polar nuclei.

The plate-like chromatic masses derived from the polar bodies (Pl. 14, fig. 6, *P.B.*[1], *P.B.*[2]), now begin to alter. Just as the fused chromosomes of the telophase of mitosis absorb a nucleoplasmic fluid and become reticulate (nesting nucleus), so do these remains of the extrusion of the polar bodies become, so to speak, blown out and frothy in shape (Pl. 14, fig. 7). The individual nucleus-like bodies derived in this way gradually become larger and finally join up (Pl. 14, figs. 7 and 8).

The original egg-shape is now finally lost, and the germinal body becomes more or less spherical. The embryonic region at the same time is not inactive, chromosomes appear in the segmentation nucleus (Pl. 14, fig. 8), and the embryonic cell divides. Only one of the daughter cells gets the germ-cell determinant—the latter going over to one cell quite intact (Pl. 14, fig. 9).

A further discrimination may now be made : The polar ooplasm and the modified remnants of the maturation divisions of the oocyte nucleus now form an investing nutrient capsule or tropho-amnion. The inner region is the embryonic part with the embryonic or germinal blastomeres (Pl. 14, figs. 9 and 10).

Pl. 14, figs. 7 and 8, mark the parting of the ways betwixt polyembryony and monembryony, and I will now describe Silvestri's case in Litomastix truncatellus, which is in certain ways intermediate between the monembryonic and the polyembryonic method of segmentation. The polar bodies are given off in the usual way, and at first are disposed exactly as in Pl. 14, fig. 6, for Ageniaspis (Encrytus).

Presently, however, the three elements approach and fuse to form a solid nucleus (Pl. 14, fig. 3 *P.N.*). The segmentation nucleus in this figure has divided, and the egg is divided into three sub-equal blastomeres. The germ-cell determinant lies in one, another has no determinant, while the third contains the polar nucleus. The two lower blastomeres are the embryonic ooplasm, the upper one the polar ooplasm. The latter subsequently gives rise to the tropho-amnion. The stage in Pl. 14, fig. 7, may now be compared with that in Pl. 14, fig. 3, and it will be noticed that in the former the method of separation of the two ooplasmas is more specialised than in the latter.

The polar nucleus in Pl. 14, fig. 3, now swells out to form a normal reticulate nucleus, which later gives rise to chromosomes and to a perfectly typical spindle. This divides, and in Pl. 14, fig. 4 the two nuclei derived from the first division are dividing again in the typical manner. In about the 8-cell stage the germ-cell determinant fragments and forms a halo around the spindle of the dividing cell which happened to receive the germ-cell determinant. This infusion of the determinant into the cytoplasm of the two daughter cells causes the latter to be somewhat conspicuous for a time (Pl. 14, fig. 4, *G.C.*, *D.C.*). Such a stage as that drawn in the latter figure is interesting, for it will be noted that the two cells containing the germ-cell determinant have entered a resting condition while their fellows around them are in the telophase of mitosis. Subsequently, however, they begin to divide again; their cytoplasm clears up, and as far as is known they form merely part of the germinal mass just as other cells do. These blastomeres containing the germ-cell determinant are later lost to sight among the other cells, and there is no evidence that they form the gonads of the future larvæ.

In Pl. 14, fig. 13, is a later stage. The polar ooplasm or tropho-amnion has many nuclei in a syncytium, while the embryonic mass has also segmented further, cell-walls being formed after each division.

The thread of the story of Ageniaspis may now be picked up again. In Pl. 14, fig. 11, is a further stage; the tropho-amnion has now become divided into an inner granular zone (endoplasm) and an outer clear zone (ectoplasm), while outside the entire germinal mass is a cellular sheath formed by the tissues of the host (*H.*).

The tropho-amniotic nucleus has constructed to form several minor polar nuclei (*P.N.*^{2}, *P.N.*^{3}), while the embryonic cells are becoming divided up into parts containing several blastomeres. These blastomeres lie in a cavity in the tropho-amniotic mass, in a fluid which in later stages is coagulable with certain fixatives.

The outer covering (*H.*) in Pl. 14, fig. 11, is of different appearance in various forms. In Martin's case it is early developed from cells of the body-cavity of the caterpillar, and is stout at first; in Marchal's case it is found developing in the same way and later forms a very definite outer sheath to the ramifying germinal mass (Pl. 14, fig. 12, and Pl. 15, fig. 15).

In the form examined specially by Silvestri, the outer or host-layer in later stages becomes very thin, much thinner than in Marchal's case (Pl. 14, fig. 14, *H.*).

In Pl. 14, fig. 13, is a later stage in Litomastix; the polar nuclei in the polar ooplasm (tropho-amnion) are now very numerous and soon will form a fairly even sheath around the embryonic ooplasmic region (E.R.).

In Pl. 14, fig. 12, a figure is given from Patterson's case in Copidosoma.

The tropho-amnion now forms a thick sheath (*T.A.*) to the inner embryonic masses (*E.R.*), while here and there adhere parts of the host fatty tissue (*F.T.*). There is also a non-nucleated inner sheath around each mass (blacked in below in Pl. 14, fig. 12) of doubtful relationship to anything described in other forms.

In all the cases examined a process begins about this time by which the spherical or ovoid germinal mass gets divided up into smaller masses. This is brought about by constrictions, primary, secondary, and tertiary, and so on, till in

some cases a ramifying figure becomes formed. The constricting process has not yet begun in Pl. 14, fig. 12, but subsequently this germinal mass will become divided up like that in Pl. 14, fig. 14. In some cases the most remarkable ramifying "sausage-like" structure such as that in Pl. 14, fig. 15, is produced, and this structure later becomes more and more attenuated, and is found to ramify throughout the whole hæmocœl. of the host caterpillar; when the parasitised caterpillar is opened one finds this peculiar body as drawn in Pl. 15, fig. 16, *P.M.* In other cases this attenuated ramifying appearance is never so marked, and the germinal mass is a shapeless, elongate body. It should be noted (Pl. 14, figs. 11 and 12) the embryonic cells are divided into parts before the tropho-amniotic layer begins to construct the mass into smaller regions.

The organ seemingly most concerned in the production of the construction of the germinal mass into smaller regions is the modified tropho-amniotic layer (Pl. 14, figs. 12 and 14), and as the constricting layer meets through the germinal masses, it carries in the outer layer derived from the host tissues (host-layer *H.*).

A word or two must now be written in connection with the structure of the tropho-amniotic layer. This sustains throughout the character of a nutrient membrane; the appearance of a heavy granulation in it is explicable on this view. Pl. 14, fig. 11, endoplasm (*E.N.P.*), and in Pl. 15, fig. 14, it will be seen that the tropho-amniotic layer around each constricted off mass is very granular and deeply-staining (*T.A.*).

In the two cases where the polar nuclei either divide by mitosis regularly (Pl. 14, fig. 13, etc.) or the polar nuclei form a frothy structure in the polar ooplasm (Pl. 14, fig. 11, etc.) the end result as far as the nuclei is concerned is nearly the same. In both cases the nuclei become very numerous either by mitoses or by amitosis, and the tropho-amniotic layer becomes well supplied with nuclei. In Patterson's case the nuclei are very even and regularly disposed as compared with

the cases described by Marchal and in one of Silvestri's forms.

Formation of Larvæ from the Inner Embryonic Masses (Marchal (8)).

The two periods of greatest interest to the embryologist in the remarkable history of this development are the stages just after the polar bodies and those of the formation of the germ-layers from the embryonic masses or gemmules.

The latter in Marchal's case were often over a hundred in number, all derived from the gemmulation of a part of the original egg.

At this period they lie inside the membranes formed by the host (*H.* in Pl. 14, fig. 11) and by the tropho-amnion (*T.A.*) and form long strings of embryonic masses or gemmules. The several scores of embryos, together with their membranes, form the germinal cord or polygerminal cordon (Pl. 14, figs. 14 and 15).

When the germinal cord is like that in Pl. 15, fig. 15, and about 3 to 4 mm. in length the gemmule or embryonic mass contains about twenty to forty cells. Each embryonic mass is really a morula, a ball of cells of the same size. During this time the cells in each morula divide constantly by mitosis, and gradually the subspherical morula becomes a distinctly ovoid mass containing sixty to eighty cells of the same general size.

From this time the embryo ceases to be represented by a simple morula, and the germ-layers commence to form. The embryo now tends to a discoid form, so that it has completely defined relations, as shown in Pl. 15, fig. 18. Its shape is reniform thanks to a depression which becomes formed in what corresponds to its dorsal region (Pl. 15, fig. 18).

The side opposite the depression corresponds to the ventral face of the embryo.

These relations are exactly the opposite of what have been described so far by other authors who have studied Chalcids or Proctotrypides.

On the convex border representing the ventral face runs a longitudinal furrow, which is not much marked in the middle region of its course, but which hollows out at the level of the stomodæum and of the posterior extremity of the body. This furrow is the representative of the mesodermal groove of other Insecta (Pl. 15, figs. 19 and 20).

Soon other furrows in a transverse direction make their appearance; these represent the incipient segmentation of the body of the embryo.

In each morula, at the largest size it attains, and at the time when the dorsal furrow (or hilus), which gives the reniform shape to the morula, begins to become hollowed out, those cells in the central region of the embryo become modified, lacunæ become formed between them, and they form a loose sort of mesenchymatous tissue.

The nuclei of these cells become very large, and the protoplasm becomes vacuolated to form a sort of network. Such modified cells form the endoderm (Pl. 15, fig. 21).

As this process has been going on the outermost cells of the morula become grouped like an epithelium, in a row. These cells constitute the ectoderm and the mesoderm, which, because of the condensation or telescoping up of the stages of development, appear to be formed synchronously Pl. 15, fig. 21).

The ecto-mesoderm, so-called, is well developed on the ventral side; in the latero-dorsal side it is not so well marked The ventral region, where the ecto-mesoderm is thickest, corresponds to the germinal band in other insects.

In short, this particular kind of development of the germ-layers in the gemmule or embryonic mass of Encyrtus is remarkable for the rapidity with which organogeny takes place, and for the manner in which the various processes are condensed, in that they occur almost synchronously. The primitive characteristics of the developmental processes of Encyrtus are masked by the occurrence of a process of delamination.

The central endoderm cells form at once the wall of the

mesenteron, and a group of yolk-cells destined to remain inside the mid-gut till the end of embryonic development (Pl. 15, fig. 21).

From the above description, which is nearly a literal translation of Marchal's work on this section of the development, it will be seen that the proctodæum and stomodæum are formed normally from invaginated ectoderm cells. The mesoderm and endoderm are formed, so to speak, in situ from the outer central and inner central regions respectively of the morula, and in this way the developmental processes of Encyrtus are remarkable.

In Pl. 15, fig. 22, is an advanced embryo showing the normal arrangement of the organs after the germ-layers are properly differentiated. This is an embryonic Litomastix, and is, in certain ways, different from Marchal's Encyrted larva (Pl. 15, fig. 21). The hilus (*h.*) is not so deep in the former, while the silk gland (*s.g.*) in Marchal's form is much more marked in this early stage.

The Independent Life of the Larvæ in the Hæmocœl. (Bugnion (3)).

In Encyrtus towards the end of June, at a time when the host caterpillars spin their cocoons, the young parasitic larvæ, now fully formed inside the tropho-amnion, break the tubular cyst in which they lie, and float free in the body-cavity. At this time also they undergo their first ecdysis, leaving the cast-off skin inside the remains of their epithelial membranes.

They are now 1 to 2 mm. in length. According to Bugnion, these larvæ for the time being are satisfied with imbibing the hæmocœlvic fluid of the host's body, but later, when they become nearly full-grown, they eat the more important organs of the host, and so kill it. Some species pupate inside, others outside the host's skin. According to Silvestri, in Litomastix there are two sorts of larvæ—sexual and asexual—derived from one polygerminal mass. The asexual larvæ are used to tear up the host tissues for the benefit of the sexual. As some doubt has been cast on this part of Silvestri's work,

nothing more need be said till this matter has been properly confirmed.

Abortive Embryos (Patterson (13)).

As has been described, the embryonic areas are derived from fragmentation of an original solid polynuclear mass. Patterson especially describes how certain of these embryonic masses are much smaller than the others, and form abortive or degenerating embryos. In some cases this production of abortive embryos seems to be due to the fact that the subdivision of the egg has been carried too far, and such embryonic masses contain too few nuclei. In other cases degeneration is apparently due to the lack of proper nutrition. Most polygerms lie in a thick layer of fatty tissue, others are nearly bare of the latter, and it is an observed fact that in such cases mortality of the embryos is very high. In one of Patterson's polygerms, with little surrounding fat tissue, there were more than 100 embryos, not more than thirty to thirty-five of which had developed normally. In Pl. 15, fig. 23 are drawn the outlines of six embryos from one caterpillar; according to Patterson, only those of the sizes from *c.* to *f.* would have developed; those of the sizes in *a.* and *b.* would degenerate.

Summary.

Before entering upon any discussion or comment on the above description of Polyembryony, a summary of the main facts known will be given.

(1) Polyembryony in the Hymenoptera parasitica is process whereby the single egg, instead of producing a single embryo, often produces several score or more.

(2) The polyembryonic Hymenoptera are generally small insects about 1 mm. in length.

(3) The polyembryonic parasite lays from one to ten or more eggs in the ovum of the host.

(4) This oviposition does not kill the host eggs. Larvæ

hatch from the latter in the normal way, but contain the eggs of the parasite, generally in the hæmocœl cavity.

(5) The parasite's egg gives off polar bodies, and may or may not be fertilised in the normal way.

(6) The polar bodies rest for a time, but then break into activity, forming an actively growing mass, or collection, of nuclei.

(7) That part of the egg cytoplasm containing the segmentation nucleus separates off from the outer part containing the active polar nuclei, and the germ-cell determinant goes to the former, but later becomes absorbed and lost to sight.

(8) The polar cytoplasm or ooplasm containing the polar nuclei forms an investing sheath around the contained embryonic ooplasm, which later gives rise to the embryos. The polar ooplasm nourishes the inner embryonic mass and acts as an amnion or placenta. Hence the name tropho-amnion.

(9) The nuclei of the tropho-amnion derived from the original polar body nuclei become very numerous by division, and the tropho-amniotic cytoplasm becomes very granular in the region of the nuclei.

(10) Certain cells of the embryo, either hæmolymph or fat cells or both, form an outer covering to the parasitic germinal mass. This host-covering later becomes much stretched and epithelial in character. In some forms it is not well developed.

(11) The primary embryonic cell separated off at the time when the polar nuclei begin to become active, has already divided many times to give rise to many germinal masses. The parasitic body lying inside the host hæmocœl, may now be called a polygerm.

(12) The polygerminal embryonic masses, keeping on dividing till as many as a hundred or more masses may be produced, later become constricted into areas each containing an embryonic mass surrounded by two membranes, the outer host-epithelial and the inner tropho-amniotic layer.

(13) The shape of the entire polygerminal mass differs in

different forms. In some it is a ramifying cylindrical body, in others a shapeless mass, constricted here and there by the outer membranes.

(14) Each separate germinal mass is now a spherical or ovoid morula containing a score or more cells. The latter keep dividing.

(15) The embryonic or germinal mass now begins to differentiate further; it loses its sub-spherical shape and becomes elongate, while dorsal, ventral, and lateral sides of the future embryo can be distinguished.

(16) The stomodæum and proctodæum are formed by invaginations of the two extreme ends of a ventral groove. The ectoderm is formed by a rearrangement of the outer cells of the morula. The endoderm and mesoderm are formed in *situ* by a modification of the more centrally-placed cells of the embryo.

(17) The larvæ at a later stage break away from their membranes and are free-living for a time. They later eat up nearly everything in the host-caterpillar's body and then pupate inside (in some cases, however, apparently outside) the body of their host.

In this review I have not so far given any data with regard to the sexes of parasites emerging from one caterpillar. Broods may be purely male or female, or mixed. In Patterson's cases 55 per cent. of all broods were female. Moreover, the average number of females emerging from a single carcase is 198 as compared with 175 for males. Some of the mixed broods doubtless arose from two or more eggs, fertilised and unfertilised; but Patterson makes the interesting suggestion that such mixed broods may also arise from a single fertilised egg by a process of disjunction of the sex chromosome during early cleavage stages (**13**). As far as is known fertilised eggs produce females, unfertilised males.

Discussion.

(For the following I alone am responsible).

The egg of the insect has been long considered one of the

most highly organised of all animals. In the ovary we can recognise its relations, where the future embryonic regions will lie and so on (18).

In no insect eggs, however, do organ-forming substances such as those found in Ascidia, appear to be demonstrable; the germ-cell determinant alone marks definitely the position of the germ-cells. Nevertheless, it is probably true that the other regions of the egg, though on a microscopical basis apparently homogeneous, are divided up into future endoderm, ectoderm, and mesoderm regions, or even into organ-forming regions. This seems directly proven by Hegner's (18) experiments with beetle eggs: "When the anterior or posterior parts of freshly-laid eggs are killed (with a hot needle) the material remaining alive develops that part of the embryo which it would have produced if the eggs had remained intact. No regeneration of the part which would have been produced by the killed region takes place." This result should be compared with what happens in polyembryony where the entire anterior end of the egg is voluntarily discarded, as far as organogeny is concerned.

It is remarkable to find that in the polyembryonic Hymenoptera a large region of the egg is entirely discarded. In fact, just that region of the egg which would have formed the head, brain, etc., of the embryo is rejected. There is no reason for supposing that the polyembryonic egg is differently organised from the monembryonic—in fact, just the reverse. The middle region, or middle region plus the lower pole of the egg, alone gives rise to the scores of embryos. We cannot recognise any special plan of division in the segmentation process; even the germ-cell determinant after passing into what in monembryonic species (15) would be the germ-cells, later becomes lost, and in view of the remarkably haphazard method of formation of the inner embryonic masses there seems little possibility that any special care is taken to ensure that the "germ-cell determinant" cells become the gonads of the future embryos. As far as we can tell, they may just as well form cells of the gut or ectoderm; Silvestri, however,

without any direct evidence, thinks that the "germ-cell determinant" cells might form the future germ-cells of each embryo.

The following conclusions I at present consider to be justified with regard to polyembryony:

(1) The "germ-cell determinant," being possibly a nutrient cytoplasmic-mass, has no other effect than that of temporarily stopping mitosis in the cells which happen to contain it. There is no evidence for supposing that the "germ-cell determinant" cells later form the germ-cells of each embryo.

(2) There is absolutely no evidence in polyembryonic species of a "germ-track"; everything is, in the first place, subservient to the production by haphazard divisions and fragmentations, of numerous morulæ, without any discoverable definite regions. Differentiation of germ-layers follows later.

(4) Mere position in the morula is all that seems to determine whether this or that cell will be an endoderm or ectoderm cell, etc.

(5) Marchal's description clearly shows that differentiation of germ-layers only takes place after the formation of the solid morula, and I can find no evidence for supposing that the inner cells differ in any way except in position from the outer cells of the morula.

From an examination of the literature on polyembryony, and from my personal knowledge of the development of a monembryonic form, I foreshadow that when more species of both monembryonic and polyembryonic forms are examined it will be found that some may be either polyembryonic or monembryonic according to season of the year, or to some condition of host egg or caterpillar. It is already known that the number of larvæ produced from one egg of different species may differ greatly; those forms with fewest larvæ from one egg are the ones in which one might expect to find transition between monembryony and polyembryony.

In certain monembryonic species the polar bodies are long

in degenerating, and, moreover, pieces of egg may live apart for a long time (15). There are still a great many parts of the polyembryonic development which should be carefully studied again; such are especially those questions connected with the segmentation of the egg, the fate of the "germ-cell determinant" cells, and the formation of germ-layers. Moreover, the "asexual" larvæ of Silvestri should be re-examined, and their true nature properly determined.

With regard to the possible place of origin of polyembryony in Hymenoptera, Marchal ('Arch. Zool. Expt. et Gener.,' 4e serie, tome iv) shows that in the monembryonic form Synopeas rhanis, the cortex of the ovum is separated in much the same way as in Pl. 14, figs. 7 and 8. The inner part of the egg, plus the segmentation nucleus, a very small part of the egg, gives rise to one embryo, instead of the many in polyembryonic forms. The cortical zone of the egg is, as far as I can ascertain from Marchal's description, supplied with nuclei from the revived polar bodies. In Synopeas rhanis the blastomeres do not part and become free in a fluid, but instead keep together, and eventually form the organs of one larva.

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EXPLANATION OF PLATES 14 AND 15,

Illustrating Mr. J. Bronté Gatenby's "Review on Polyembryony in Parasitic Hymenoptera."

Explanation of Lettering.

A. Amnion of host caterpillar. *B.L.* Separated blastomeres of polyembryonic egg. *br.* Brain. *CH.* Chromosomes. *coag.* Fluid in which embryonic blastomeres lie and which coagulates in fixatives. *D.* Dorsal. *ECTP.* Ectoplasmic or clear region of polar ooplasm.

E.M. Embryonic mass. *END.* Endoderm. *ENP.* Inner or granular region of polar ooplasm. *ER.* Definitive embryonic region of egg. *F.* Space filled with fluid. *FC.* Adipose tissue cell of host. *F.PN.* Female pronucleus. *G.* Gut of host caterpillar. *G.C.D.* Germ-cell determinant. *G.C.D.C.* Cell which contains germ-cell determinant. *G.L.* Granular layer around each embryonic mass. *H.* Layer of cells around the polyembryonic mass formed by cells of the host. *h.* Hilus or dorsal depression in the kidney-shaped larva. *Lit.* Litomastix imago ovipositing in ova of Plusia. *M.PN.* Male pronucleus. *N.ch.* Nerve cord. *PD.* Proctodæum. *P.E.* Egg of host moth, Plusia. *P.M.* Parasitic mass or polygerminal cordon in hæmocœl of Plusia caterpillar. *P.N.* Polar nucleus. *P.OP.* Polar ooplasm. *SP.* Spermatozooni *st.* and *STD.* Stododæum. *TA.* Tropho-amnion, or polar ooplasm. *TU.* Tracheal tube of host larva. *V.* Ventral. *Z.N.* Zygote nucleus.

PLATE 14.

Figs. 1–4. (All after Silvestri.)

Fig. 1.—Litomastix truncatellus (Dalm.) in the act of ovipositng in the egg of a moth. Plusia gamma. L. (Much enlarged.) *Lit.* = Parasite. *P.E.* = Plusia egg.

Fig. 2.—Section through egg of Plusia containing an embryo nearly eady to hatch. The substance of the host (Plusia) caterpillar contains many Litomastix eggs. Three lie in the mouth and stomodæum of the caterpillar and will probably never hatch out. One lies just behind the brain (*Br.*), two more lie beneath the nerve-chord, while another has been oviposited outside the body of the host.

Fig. 3.—Three-cell stage in the segmentation of the egg of Litomastix. The three polar bodies (*P.N.*) (compare fig. 6) have now fused to form a single solid mass of chromatin; no other nucleus lies in this upper blastomere or polar ooplasm (*P.O.P.*). One of the lower blastomeres contains the germ-cell determinant (*G.C.D.*), the other only a segmentation nucleus (*B.L.*^{2}). (Four comp. eye-piece, $\frac{1}{12}$ semi-apocromat., camera lucida.)

Fig. 4.—Same species as in last figure, but at 8 blastomere stage. The two cells which received the germ-cell determinant (*G.C.D.C.*) are temporarily resting. The polar body had divided once, and in the present figure the two cells derived from the first mitotic division are themselves now dividing by mitosis. (Drawn in same way as before.)

Figs. 5–10. (All after Martin.)

Fig. 5.—Egg of Ageniaspis. The first polar body (*P.B.*^{1}) has been given off and is dividing again; the second polar body is being formed, the upper part of this spindle being the second polar body, the lower

part the female pronucleus. The egg contains a sperm (*SP.*) and a "germ-cell determinant" (*G.C.D.*).

Fig. 6.—Later stage, polar bodies given off; the pronuclei, male (*M.PN.*) and female (*F.PN.*) are about to unite. The "germ-cell determinant" lies near (*G.C.D.*).

Fig. 7.—Zygote nucleus formed (*Z.N.*), the polar bodies have begun to form nuclei (at *P.N.*), and the polar ooplasm (*P.OP.*) is separated by a space from the embryonic ooplasm or region (*E.R.*). The embryonic ooplasm contains the zygote nucleus and the "germ-cell determinant."

Fig. 8.—Polar nuclei fusing to form the tropho-aminotic nucleus (*P.N.*); the chromosomes are appearing in the segmentation nucleus (*CH.*).

Fig. 9.—The embryonic nucleus has divided by mitosis to form two cells, only one of which contains any "germ-cell determinant"; the complete fusion of the secondary polar nuclei has taken place to form a mass in the polar ooplasm (*P.OP.*) or tropho-amnion. The elongate shape of the egg is being lost.

Fig. 10.—The embryonic cells are now three in number, one of which contains the germ-cell determinant. The tropho-amniotic or polar nucleus is becoming compressed before it begins to divide into regions by amitosis. The three blastomeres are not adherent to one another.

(All figures drawn with camera lucida eye-piece 4 and a $\frac{1}{12}$th o.im. Slightly reduced.)

Fig. 11.—Polygerm or multiple germinal mass at later stage. Polar or tropho-amniotic nuclei divided into three (*P.N.*^{1} to *P.N.*^{3}). The three blastomeres in fig. 10 have divided to form nine cells. The polar ooplasm now has an outer clear zone, ectoplasm (*ECTP.*), and an inner granular zone, endoplasm (*ENP.*), Outside the polar ooplasm or tropho-amnion is a membrane (*H.*) derived from cells of the body-cavity of the host caterpillar. (At about same magnification as previous figures; after Martin.)

Fig. 12.—Polygerm (after Patterson) of Copidosoma, showing end phase of embryonic-region formation. At *F.* are fat cells of embryo, at *coag.* a precipitate, at *G.L.* a granular layer (purposely blacked in below to show it), and at *TA.* the tropho-amniotic layer. (Nucleated membrane, polar ooplasm.) × 480, somewhat reduced from original.

Fig. 13.—Litomastix germinal mass, to follow stages in figs. 3 and 4. Polar nuclei now very numerous. Embryonic nuclei (*E.R.*) very numerous, germ-cell determinant cells lost among other cells of embryonic region. (After Silvestri.)

PLATE 15.

Fig. 14.—Polygerminal mass of Litomastix in optical section, showing granular tropho-amniotic membrane (*T.A.*), embryonic regions (*E.R.*), each of which gives rise to an embryo, and the outer host-membrane (*H.*). (After Silvestri.)

Fig. 15.—Embryonic chain or cordon (polyembryonic mass) of Encyrtus, all derived from one egg. *T.U.* Tracheal tube of host. × 63. (After Marchal.)

Fig. 16.—Larva of Hyponomeutus opened and containing several chains of Encyrtus (*P.M.*). × 3. (After Marchal.)

Fig. 17.—Part of the same preparation as that in fig. 15, more magnified, showing inner embryonic masses (*E.R.*), tropho-amnion (*T.A.*), and outer membrane (*H.*), derived from host.

Fig. 18.—Embryonic mass from side after assumption of reniform shape. The hilus (*h.*) of the "kidney" represents the dorsal surface (*D.*) of the embryo: at *st.*, just above the hilus, is the stomodæl depression. × 159.

Fig. 19.—Advanced embryo viewed in profile from the left side: the hilus (*h.*) is the dorsal side, at *st.* is the stomodæum, at *pd.* the proctodæum, and signs of external segmentation are appearing. *V.* Ventral side. *D.* Dorsal. × 159.

Fig. 20.—Same stage viewed from dorsal face. *st.* and *pd.* as before. × 159.

Fig. 21.—Slightly oblique longitudinal sagittal section of an embryo such as that in figs. 19 and 20. At *sg.* is the silk (salivary) gland; at *st.* the rudimentary stomodæum; at *pd.* the proctodæum; at *h.* the hilus. The endoderm consists of the inner large scattered cells (*end.*). The inner edge of the ectoderm (*ect.*) is not clearly defined as yet, and many of the cells at *m.*, which are forming the mesoderm series of organs, are still intercontinuous with ectoderm cells. At *x.* is a region where cells might be ectoderm, endoderm, or mesoderm, their true nature not yet properly defined. × 340.

(All these figures after Marchal.)

Fig. 22.—Later stage of embryo of Litomastix after Silvestri. Nearly all organs clearly defined. *st.*, *pd.*, *end.*, *m.*, *D.* and *V.* as before. *br.* Brain; *nch.* nerve cord; *mal.* malpighian tubule.

Fig. 23.—*a.* to *f.*, six Copidosoma larvæ free in the body-cavity of same host, to show great variation in size of larvæ from one caterpillar. (After Patterson.)